Significance of Plasma Lead Levels in Normal and Lead-Intoxicated Children*

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Plasma lead (Pb) levels have been measured in normal and lead-intoxicated children, newborns, and children with sickle cell disease. The results in all groups were contant over a wide range of red cell Pb concentration. These results support the thesis that *the red cell represents a large repository for Pb, maintaining plasma Pb concentration within closely defined limits, and that methods other than measurements of plasma Pb will be necessary to uncover a presumably dynamic transport system between red cell and plasma.* Indeed, we have demonstrated *in vitro* that ionized calcium (Ca^{2+}) lowers red cell Pb content according to a linear dose–response curve. Ca^{2+} may thereby control Pb transport from red cell to plasma, and fluctuations in the concentration of Ca^{2+} in serum and extracellular fluid may influence the toxic activities of Pb. In bone organ culture, changes in the concentration of Ca^{2+} and phosphate in the medium alter the release of previously incorporated ^{210}Pb from fetal rat bones in response to parathyroid hormone (PTH). Therefore, both PTH and the ionic milieu of the medium apparently regulate bone Pb metabolism.

We would expect that understanding further the dynamics of Pb transport in plasma and bone may lead to a more exact definition of the real hazards of low level Pb toxicity in children.

Our studies, focusing on the metabolism of lead in bone and plasma, hopefully, will help to define more clearly the dynamics of lead transport in children. By so doing, perhaps the real and potential hazards of low level lead (Pb) toxicity may be understood, and perhaps, more effective therapy may evolve for the treatment of childhood lead intoxication.

Plasma

In previous reports, the concentration of

plasma Pb has been summarized as varying from 0 to less than 5 μg/100 ml in lead-intoxicated children (1–3), though the specific data supporting the latter statement have rarely, if ever, been published. Recently, McIntyre and Angle (4), on the basis of theoretical regression lines, have estimated Pb concentration in serum (or plasma) of a magnitude several times greater than previous estimates. In addition, these authors indicated that serum Pb levels were lower in anemic children deficient in glucose-6-phosphate dehydrogenase compared to nondeficient children. To examine the usefulness of Pb levels in plasma as an indicator of Pb metabolism, and to evaluate possible differences in the partition of Pb in low

and high hematocrit blood, we have measured directly plasma Pb levels in 165 children.

Measurements of lead were carried out on 1.0 μl aliquots of plasma, according to a metric technique with the carbon rod atomizer (CRA), as previously described (5). The sample, contained entirely within a disposable plastic tip, was injected into a model 63 CRA with a micropipettor (Unimetrics #8010M), and was sequentially dried, ashed, and atomized according to the preset voltage/time cycles on the power supply. The analytical line was 2170 A. Standards were prepared by diluting the working stock solution with appropriate volumes of a mixed salt solution that contained, per liter, 0.48 g of KH_2PO_4, 0.28 g of $CaCl_2$, 2.90 g of NaCl, 0.07 g K_2SO_4, and 8.10 g of EDTA (disodium salt). These standards, closely approximating the ionic matrix of plasma, were used to account for ionic interferences present in plasma.

The sensitivity was 0.35 μg Pb/100 ml, and the standard deviation of 165 determinations was 0.92 μg Pb/100 ml. Recoveries of Pb at concentrations of 1, 4, and 7 μg/100 ml, when added to plasma, averaged 0.8, 4.1, and 7.2 μg/100 ml, respectively. Experiments with radioactive ^{210}Pb yielded similar recoveries (6).

The results have shown a remarkable constancy in Pb concentration in the plasma of children over a wide range of hematocrit and whole blood Pb levels (Table 1) (6). No significant statistical difference in these plasma values could be established for children with sickle cell disease, lead intoxication, or for newborns, when compared to normal children (Table 2). In brief, similar to an earlier study of adult human blood in vitro (7), these data indicate that levels of lead in plasma remain constant in the face of wide differences in the concentration of lead in red blood cells. Though these results have agreed, generally, with two preceding reports, very few subjects previously reported fell within the pediatric age group; and none of these had either symptomatic

Pb intoxication and/or sickle cell disease (2, 3).

These observations have provided further support for the hypothesis that the red cell represents a rich repository for Pb (8), maintaining plasma Pb levels within very closely defined limits. It may well be that only a small fraction of lead in plasma is in a physiologically active form; and total plasma lead would exhibit very small changes, not detected even by this sensitive nonflame atomic absorption method, as blood lead rises. Our own studies of normal adult plasma have indicated that a relatively small, but demonstrable amount of total plasma lead can be ultrafiltered through a membrane with a molecular weight cut-off of 2000 (Rosen and Trinidad, unpublished observations). It appears, therefore, that the binding capacity of red cell receptors for lead may be relatively unlimited, at least within the range of blood Pb values reported herein. Accordingly, correction of whole blood Pb levels for hematocrit appears not to be warranted. Thus, recalculation of whole blood Pb levels for hematocrit fails to provide a more precise index of Pb toxicity, and fails to reflect the likely biochemical realities involved in the partition of Pb in whole blood. Our data are consistent with the postulate that phosphate (7) and perhaps other binding sites (9–11) of red cells are critical biochemical factors in the partition of lead in blood—not red cell mass per se; dynamic methods (12), other than an isolated measurement of plasma Pb, will likely be necessary to uncover mechanisms of lead transport between red cell, plasma, soft, and hard tissues.

Indeed, we have demonstrated now in vitro that ionized calcium (Ca^{2+}), at a concentration of 4 mg/100 ml or greater, displaces lead from "leaded" rat erythrocytes according to a linear dose response curve (Fig. 1) (12). In this system, red cells were collected from lead-intoxicated rats, washed, and suspended in a Ca^{2+}-free chemically defined medium at pH 7.40. Varying concentrations of Ca^{2+}, as $CaCl_2$, were added to the cell suspensions, which were then incubated at

Table 1. Results of plasma lead determinations, whole blood lead concentration, and hematocrit in all patient groups.

	"Normals"		Undue exposure likely			Undue exposure certain				Children with sickle cell disease	Newborns
Range of whole blood Pb, μg/100 ml [a]	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99	100–136	18–136	12–34; (mean, 19)
Number of subjects	14	17	24	24	14	14	10	8	10 [b]	17 [b]	13
Mean plasma Pb, μg/100 ml ± 1 S.E.M.	3.0±0.44	3.2±0.33	3.0±0.37	3.3±0.41	3.2±0.34	3.10±0.31	3.0±0.30	3.0±0.33	3.2±0.39	3.1±0.35	3.0±0.40
Range plasma Pb, μg/100 ml	1–7	1–6	1–7	1–7	1–5	1–7	2–4	1–3	1–5	1–6	1–4
Mean hematocrit, %	36	36	34	34	33	31	29	28	26	20	60
Range of hematocrit, %	35–37	35–37	34–36	34–36	31–35	30–33	28–30	27–30	25–28	18–24	48–75

[a] Whole blood Pb levels have not been corrected for hematocrit.
[b] These groups include patients with encephalopathy.

Table 2. Summary of data.

Subject groups [a]	Mean plasma Pb, μg/100 ml ± S.E.M.
"Normals" (20–39 μg/100 ml)	3.10±0.31
Undue exposure likely (40–59 μg/100 ml)	3.20±0.29 [b]
Undue exposure certain 60–136 μg/100 ml	3.10±0.26 [b]
Children with sickle cell disease (18–136 μg/100 ml)	3.10±0.28 [b]
Newborns (12–34 μg/100 ml)	3.0±0.31 [b]

[a] Values in parenthesis indicate whole blood Pb concentration, not corrected for hematocrit.

[b] None of these values differ significantly from those for the "normal" group.

37°C for 30 min. The concentration of Ca^{2+} was measured by an ion-specific electrode, ^{210}Pb was counted on an auto-gamma spectrometer, and stable Pb was measured on the CRA.

After incubation with Ca^{2+} in final concentrations of 0, 2, 4, 6, and 8 mg/100 ml, red cell Pb decreased by 0, 0, 12, 24, and 40%, respectively (Fig. 1). These highly significant decreases in red cell Pb, only above a threshold concentration of 4 mg/100 ml, were complete within 30 min (refer to Fig. 2 for description of *in vitro* red cell system). Experiments with ^{210}Pb yielded similar results.
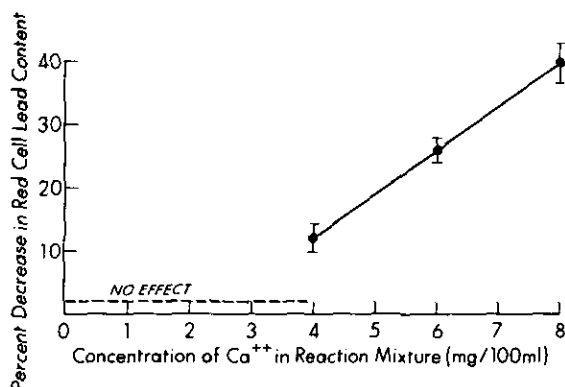

FIGURE 1. Linear dose-response curve that demonstrates displacement of erythrocyte Pb by Ca^{2+}, according to the *in vitro* red cell system outlined in the text. The decreases in red cell Pb content at Ca^{2+} concentrations of 0, 2, 4, 6, and 8 mg/100 ml, respectively, represent the mean (in per cent) ± 1 S.E.M. of six experiments at each concentration of Ca^{2+}.

Furthermore, Zn^{2+}, Cu^{2+}, and Mg^{2+}, up to 1.00, 1.00, and 2.5mM, respectively, had no effect on the concentration of red cell Pb. Moreover, in this system, red cell compartments of lead appear heterogeneous, i.e., below an erythrocyte lead level of 35 μg/100 ml, Ca^{2+}, even above its concentration threshold, does not displace red cell-bound Pb (Rosen and Trinidad, unpublished observations).

Two lines of evidence suggest that this phenomenon represents a specific action of Ca^{2+} on the erythrocyte membrane, with the result that lead–salt complexes (presumably lead triphosphate) are displaced from the red cell, and calcium ion effectively competes with Pb for these salts: (1) the concentration of calcium ion, once introduced into the reaction mixture, rapidly decreases in the same ratio as Pb does, and (2) calcium glycerophosphate, a nondissociable
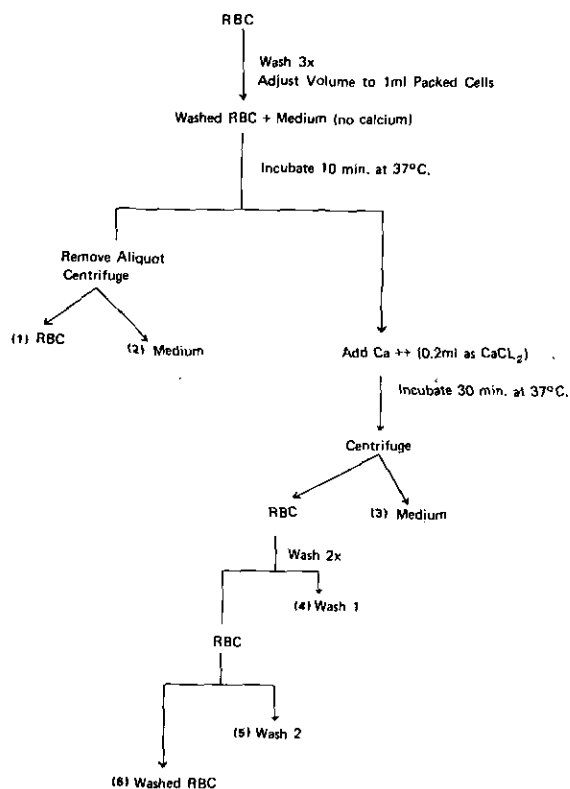

FIGURE 2. In this *in vitro* red cell system, the concentration of red cell Pb (1, no Ca^{2+} in the system) is measured and compared to the level of red cell Pb (6) with Ca^{2+} in the system.

salt at pH 7.40, had no effect on red cell lead content in this system (Rosen and Trinidad, unpublished observations.) These data suggest, therefore, that Ca^{2+} may control Pb transport from red cell to plasma; and changes in the concentration of Ca^{2+} in serum and extracellular fluid may influence both the dynamics of Pb transport and the toxic activities of Pb.

The *in vivo* correlates of these *in vitro* observations are notable (13–16). In lead-intoxicated rats, challenge with Ca^{2+}, above a specific concentration threshold comparable to the one *in vitro*, produces a rapid decrease in red cell Pb content; this effect is maintained after levels of Ca^{2+} in serum return to baseline values. Moreover, similar to the *in vitro* system, below an initial concentration of about 40 μg/100 ml, a decrease in erythrocyte lead concentration cannot be demonstrated (17). Furthermore, in six Pb-intoxicated children, levels of ionized calcium in serum increased 1.50 mg/100 ml ±0.2 (mean ± S.E.M.) above baseline values, during chelation with EDTA (calcium disodium salt (Rosen and Trinidad, unpublished observations). These increases closely parallel the fall in blood Pb levels; and the return of Ca^{2+} in serum to baseline values coincides with an increase in blood Pb content shortly after the end of EDTA therapy. Hence, part of the observed EDTA effect in these children may be secondary to the effects of calcium ion administered with EDTA.

Bone

The skeleton is the ultimate reservoir for over 90% of the human body burden of Pb. In children, the factors that control Pb metabolism in bone are particularly critical, since bone represents the major source of Pb mobilized by EDTA. In addition, "subtoxic" amounts of Pb may be mobilized during the course of rapid skeletal growth in children, a process involving concurrently new bone formation and bone resorption. Presumably, the cardinal factors effecting bone mineral mobilization, namely, calcitonin, PTH, vitamin D metabolites, 3',5'-

AMP, and the ionic composition of the extracellular fluid, control the efflux of Pb from bone.

We have begun recently to study the skeletal metabolism of Pb in bone organ culture. To increase the amount of stable Pb in fetal rat bones, pregnant rats are intoxicated with Pb throughout prgenancy by adding lead acetate to the drinking water. On the 17th day of gestation, 50 μCi of ^{210}Pb in 2 mg of carrier Pb are injected IV into each pregnant rat. By the 18th day of gestation, blood Pb levels in the pregnant rats have reached 40–60 μg/100 ml. At this time, when paired radii and ulnae are dissected and "planted" on grids in modified BGJ medium, the amount of Pb in these fetal bones is four to six times that in normal fetal bones. After 2–4 days in culture, the radioactivity released from treated bones to the medium is compared to that released from paired control bones. ^{210}Pb activity in the medium is measured in a liquid scintillation spectrometer. In these experiments, the concentrations of ions and protein in the medium have been modified to closely approximate those in the extracellular fluid (18).

Early results in this system are (1) increasing the concentration of phosphate (1.0 to 5.0mM) or Ca^{2+} (1.5 to 3.00mM) produces a significant decrease in lead efflux of 60 and 24%, respectively, in response to PTH (18); (2) changes in the concentration of Mg^{2+} in the medium have no effect on the efflux of ^{210}Pb in response to PTH; (3) the magnitude in efflux of ^{210}Pb from bones treated with PTH becomes considerably greater as the initial concentration of stable Pb rises. These results suggest that PTH and the ionic milieu of the medium (extracellular fluid) interact and exert considerable control on the release of previously incorporated ^{210}Pb and that the initial concentration of stable Pb is critical in evaluating the magnitude in efflux of ^{210}Pb from explant to medium. After an extensive number of Pb analyses of ashed bones, the concentration of stable Pb and the magnitude in efflux of ^{210}Pb have become sufficiently reproducible

now in control versus PTH-treated bones (Rosen and Trinidad, unpublished observations).

In summary, we expect that understanding further the dynamics of Pb transport in plasma and bone may lead to a more exact biological definition of the real and potential hazards of low level Pb toxicity in children.

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